

The present claim amendments are fully supported by the application including the Drawings and claims as filed originally. A clean copy of page 13 of the specification (as amended) is enclosed herewith.

Turning to the Office Action at pg. 2, Applicant has been requested to cancel certain claims. The request is addressed by this submission.

The specification was objected to on pg. 13, line 9. The objection is a matter of form and has been addressed by this submission.

The claim objection discussed at pg. 2, last line of the Office Action has been addressed by this submission.

Claims 1-5 stand rejected under 35 USC §112, first paragraph on ground that the specification "does not reasonably provide enablement for using the method with p53". At pg. 3. While Applicant respectfully disagree with this position for reasons already of record, basis for the rejection has been addressed. Claim 1 has been amended.

It is noted that the specification as filed shows how to make and use a variety of tumor-associated antigens (TAAs) in accord with the instant invention eg., Her-2/neu, RAS, tyrosinase, MART, Gp100, Mage, Bage and MUC-1.

In view thereof, reconsideration and withdrawal of the rejection are requested.

Claims 1-5 stand rejected under 35 USC §112, second paragraph, on grounds that certain claim phrases are indefinite. While Applicant must disagree with each position taken at pg. 4 of

the Office Action, grounds for each basis of rejection have been addressed. Particularly, each of the phrases referenced in rejection at pg. 4 have been deleted.

As to the rejection of claim 4, Applicant respectfully disagrees. However basis for the rejection has been addressed by this submission. Claim 4 has been amended.

With respect to claim 1 (as amended), the featured nucleic acid encodes at least one of each of the variable regions of the  $\alpha$  and  $\beta$  chains. That is, the nucleic acid must include one of the  $\alpha$  chain variable regions and one of the  $\beta$  chain variable regions.

Claims 1-5 stand rejected as unpatentable over the Man reference (*J. of Immunology*, 153: 4458-4467 (1994)) in view of Cole (*FASEB J.* 9: pg. A801 (1995)). Applicant respectfully disagrees with the rejection for reasons already of record. Applicant further disagrees with the Office in view of the present submission.

Claim 1 has been amended to include a further step in which the receptor-encoding nucleic acid molecules are fused together. The recited nucleic acid molecule thus encodes a one chain non-human T-cell receptor (TCR). Additionally, encoded TCR includes at least one of the variable regions of the alpha chain and at least one of the variable regions from the beta chain. See amended claim 1. Applicant's method of making such a nucleic acid is not taught or suggested by the cited references either taken individually or together with the other references of record.

For instance, the cited Man and Cole references are understood to disclose methods by which multiple nucleic acids are used to make two chain molecules. In contrast, the present invention methods fuse nucleic acids to make a one chain molecule. Thus the methods disclosed by the Mann and Cole references, as relied on, are different from the method of claim 1. Accordingly, there is no basis for maintaining the present obviousness rejection.

More specifically, the cited Man and Cole references do not teach or suggest any fusion step for making the claimed nucleic acid molecule. That is, the claimed invention includes a fusion step that joins the receptor encoding nucleic acid molecules together. That fusion step is not disclosed or suggested by the references as relied on in the rejection. The claimed invention is thus different from the cited methods which report manipulation of nucleic acids as separate chains to make heterodimeric (multiple chain) TCRs. In light of this important difference, there is no basis for maintaining the obviousness rejection.

Further, the cited Man and Cole references do not provide any motivation to fuse nucleic acids together as Applicant has done in the claimed methods. At best, the references describe routine manipulations intended to make the heterodimeric TCRs. In contrast, the claimed invention includes a fusion step that joins nucleic acid molecules together as one chain. The result is the claimed nucleic acid which encodes at least one of each of the variable regions of the alpha and beta chains. See amended claim 1. As cited, the references do not provide any motivation to include Applicant's fusion step.

In view thereof, the Applicant requests that the obviousness rejection be reconsidered by the USPTO and withdrawn.

Enclosed with this submission is a Notice of Appeal along with the requisite appeal fee.

Although it is not believed that any fee is needed to consider this submission or the enclosed Notice of Appeal, the Office is hereby authorized to charge such fee(s) to our Deposit Account No. 04-1105 if it is deemed necessary.

If the undersigned can be of any assistance in expediting the prosecution of this application, or if there are any questions concerning the above submission, the Examiner is encouraged to call the undersigned collect at the number given below.

Attached to this submission is a marked-up version of the changes made to the specification and claims. The attached page is captioned "version with markings to show changes made".

Respectfully submitted,

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PATENT TRADEMARK OFFICE

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**IN THE SPECIFICATION:**

Paragraph at pg. 13, lines 7-10, has been deleted and the following paragraph has been inserted:

A preferred vector for the insertion of the modified sequences, pBJ1Neo with a polylinker insertion site is shown in Figure 8. The host vector, pBJ1Neo is described in [\_\_\_\_,] *Mol. Cell Biol.* (1988) 8: 466; the polylinker is described in [by \_\_\_\_\_, ] *Science* (1990) 249: 677.

**IN THE CLAIMS:**

Claims 6-21 have been cancelled without prejudice.

Claims 1 and 4 have been amended as follows:

1. (Amended) A method to prepare an isolated nucleic acid molecule having a nucleotide sequence encoding at least one of each of the variable regions of [each of ] the  $\alpha$  and  $\beta$  chains of a non-human T-cell receptor (TCR) which TCR is specific for a tumor-associated antigen (TAA) [selected from Her-2/neu, RAS, p53, tyrosinase, MART, Gp100, Mage, Bage and MUC-1,] which method comprises

immunizing a transgenic non-human mammal species, which produce human HLA, with an effective amount of said TAA to produce HLA restricted cytotoxic T lymphocytes (CTL) which display TCR specific for said TAA in amounts sufficient to lyse tumor cells having the TAA,

recovering said HLA restricted CTL, which contain said nucleic acid molecules encoding at least one of each of the variable regions of the  $\alpha$  and  $\beta$  chains of a non-human TCR,

cloning or amplifying said nucleic acid molecule encoding the TCR nucleotide sequence isolated from the HLA restricted CTL<sub>2</sub> [and]

recovering said TCR receptor-encoding nucleic acid molecules; and

fusing the recovered nucleic acid molecules together to prepare the isolated nucleic acid molecule, wherein the fused nucleic acid molecules encode a single-chain TCR.

4.(Amended) The method of claim 3 wherein the cloning or amplifying step further comprises a polymerase chain reaction using primers derived from murine TCR [is used to amplify said nucleic acid molecule].

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$\beta$  chains of the TCRs recovered from CTLs recovered in mice that had been administered the H7 peptide.

Chimeric molecules similar to those described hereinabove for clone 4 and as set forth in Figures 1 and 2 were prepared from the amplified sequences of the H7-specific  
5 RR functionality is assayed by transfecting MD45.27 and testing for the production of IL-2 as described hereinabove.

A preferred, vector for the insertion of the modified sequences, pBJINeo with a polylinker insertion site is shown in Figure 8. The host vector, pBJINeo is described in  
10 *Mol Cell Biol* (1988) 8:466; the polylinker is described *Science* (1990) 249:677.

The dimer and single chain constructs were transfected into 27J cells and the cells measured for production of IL-2 in the presence of JA<sup>2</sup> cells plus H7 peptide. As shown in Figure 9, all transfectants produced with the H7 specific TCR derivatives produced IL-2. 27J cells without these constructs did not produce IL-2 in response to the JA2 cells and  
15 peptide, and none of the cells produced IL-2 in response to JA2 cells alone.

Finally, Figure 10 shows the production of IL-2 by these four constructs transfected into 27J cells in response to HER 2/neu derived peptides and cells presenting such peptides. Again, all four constructs rendered the transfected cells responsive.

#### Example 4

##### 20 Preparation of T cells Expressing TCR and its Derivatives

Human PBL that are CD8<sup>+</sup> are transduced with the chimeric constructs described above using the LXS<sub>N</sub> and LXSH retroviral vectors (Hock, R.A. *et al. Nature* (1986) 320:275) and the technique of Anderson, W.F. *Science* (1992) 256:808. The  $\beta$  chimeric gene is inserted into the LXSH retroviral vector which confers Hygromycin B resistance  
25 and  $\alpha$  chimeric gene in LXS<sub>N</sub> retroviral vector which confers neomycin resistance; thus selection of T lymphocytes expressing both the V  $\alpha/\zeta$  and V  $\beta/\zeta$  can be recovered. Recombinant retrovirus-producing cell lines are generated by transfection of the vectors  
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